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Divergent Prolactin and Pituitary-Adrenal Activity in Rats Selectively Bred for Different Dopamine Responsiveness*

NYNKE Y. ROTS, ALEXANDER R. COOLS, MELLY S. OITZL, JEANNETTE DE JONG, WIN SUTANTO, AND E. RONALD DE KLOET

Division of Medical Pharmacology, Center for Bio-Pharmaceutical Sciences, Leiden/Amsterdam Center for Drug Research, University of Leiden, 2300 RA Leiden; and the Department of Psychoneuropharmacology, University of Nijmegen (A.R.C.), Nijmegen, The Netherlands

ABSTRACT

The present study explores the significance of brain dopamine phenotype for individual variation in the neuroendocrine stress response of the rat. For this purpose, we used two Wistar rat lines previously selected for high or low responsiveness of the dopamine system to apomorphine using the gnawing response as the selection criterion. Systemic administration of the drug evoked in apomorphine-susceptible (apo-sus) rats a vigorous gnawing response, whereas apomorphine-unsusceptible (apo-unsus) rats did not gnaw under these conditions. These two rat lines represent individuals displaying extreme differences in gnawing behavior that otherwise coexist in a normal Wistar population. In this study basal and stress-induced hypothalamic-pituitary-adrenal activity and PRL release were measured in chronically cannulated, freely moving rats that endured a conditioned emotional response. Tyrosine hydroxylase messenger RNA (mRNA), corticosteroid receptor mRNA, and *in vivo* retention of [³H]corticosterone were measured in rat brain sections using *in situ* hybridization and *in vivo* autoradiography.

The results show that 1) apo-sus rats had a markedly reduced PRL response to stress compared to apo-unsus animals, whereas basal levels were not significantly different. A12 dopaminergic neurons in the arcuate nucleus expressed significantly higher levels of tyrosine hydroxylase mRNA in apo-sus rats, suggesting that the reduced stress-induced PRL release could be due to an increased inhibitory

control by dopaminergic neurons; 2) in apo-sus rats, stress resulted in a sustained elevation of ACTH and free corticosterone levels, whereas the total corticosterone levels were not different between the two rat lines; 3) under basal morning conditions, apo-sus rats had significantly higher plasma ACTH, but, in contrast, lower free corticosterone than apo-unsus rats; total plasma corticosterone levels were not different; 4) the basal evening ACTH level was elevated in apo-sus rats; after removal of the adrenals in the morning, this increased ACTH level in apo-sus rats persisted into the afternoon 6 h postadrenalectomy; and 5) hippocampal mineralocorticoid (MR), but not glucocorticoid (GR), receptor capacity was increased in apo-sus rats, with the affinity of both receptors for the ligand comparable between the groups; the MR of apo-sus rats displayed an increased retention of [³H]corticosterone in all hippocampal cell fields measured 24 h after adrenalectomy; MR and GR mRNA in hippocampus as well as GR mRNA in the paraventricular nucleus were not significantly different in the two rat lines.

In conclusion, the data suggest a common genetic background for individual variation in stress responsiveness and dopamine phenotype. High dopamine reactivity is linked to a reduced PRL and an increased ACTH response after stress. These high dopamine responders display a hyporesponsive adrenal cortex and corticosteroid feedback resistance associated with altered brain corticosteroid receptor properties. (*Endocrinology* 137: 1678–1686, 1996)

RECENT STUDIES have shown that dopamine is linked to stress. For instance, stress induces a PRL response, and this release of PRL is under inhibitory control of dopamine (1–3). Furthermore, rats with a predisposition to develop amphetamine self-administration display prolonged adrenocortical activation and increased locomotor activity after exposure to the stress of novel environment (4–6). Glucocorticoids promote activation and sensitization of the ascending dopaminergic neurons. Moreover, dopaminergic activation of CRH neurons may occur (7, 8). The data add to a growing body of evidence suggesting adverse effects of aberrant corticosteroid hormone signaling on dopamine-dependent psychopathology, including psychoses precipitated by excess glucocorticoids and stress. The susceptibility

to stress and dopamine displays a pronounced individual variation.

The present study was designed to test the hypothesis that individual differences in dopamine and stress responsiveness are linked by a genetic background. For this purpose, we used two rat lines genetically selected from a normal Wistar population on the basis of their susceptibility to the dopamine agonist apomorphine (9). Systemic administration of the drug produced very high gnawing scores in animals designated the apomorphine-susceptible rats (apo-sus). Low gnawing scores were measured in other rats, *i.e.* the apomorphine-unsusceptible rats (apo-unsus). The rat lines appear distinct in behavioral adaptation. After the psychochallenge of defeat, apo-sus rats showed a fleeing response rather than the freezing response shown by apo-unsus rats (9). This finding suggests that the two rat lines represent rats displaying extreme differences in coping with stress that coexist in an unselected population.

Previous observations showed line differences in hypothalamic CRH messenger RNA (mRNA) level and ACTH release during exposure to novelty (10). These findings imply

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Address all correspondence and requests for reprints to: Prof. Dr. E. R. de Kloet, Division of Medical Pharmacology, Leiden/Amsterdam Center for Drug Research, Sylvius Laboratories, P.O. Box 9503, University of Leiden, 2300 RA Leiden, The Netherlands.

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that apo-sus rats may be more resistant to corticosteroid action, possibly due to differences in dopamine-dependent central drive and/or altered corticosteroid receptor properties (10, 11). In view of the preliminary observations that implied changes in the neuroendocrine stress response system associated with the degree of apomorphine susceptibility, we focused on the following objectives in a comparative study of the two rat lines. First, the tyrosine hydroxylase (TH) mRNA level in A12 tuberoinfundibular neurons and the level of circulating PRL were measured. Second, basal and stress-induced hypothalamic-pituitary-adrenal (HPA) activities were assessed in terms of analysis of ACTH and corticosterone patterns in blood obtained by sequential blood sampling from freely moving rats. Third, mineralocorticoid (MR) and glucocorticoid (GR) receptors were measured in hippocampus and hypothalamus by *in vivo* labeling and subsequent autoradiography of brain sections, *in vitro* cytosol binding, and *in situ* hybridization. We found that genetic selection for extreme differences in dopamine phenotype results in individuals showing strongly divergent patterns of PRL release and pituitary-adrenal activity that otherwise coexist in a normal unselected population.

Materials and Methods

Animals

Adult male Wistar rats of two pharmacogenetically selected rat lines, weighing 120–140 g at arrival in Leiden, were obtained from the Department of Psychoneuropharmacology, University of Nijmegen (Nijmegen, The Netherlands). Selection of the two rat lines was based on their responsiveness to the dopamine agonist apomorphine. Rats of one line, the apo-sus line, are highly susceptible for apomorphine-induced gnawing, whereas rats of the apo-unsus line show only a minimal gnawing response.

The selection procedure was described in detail by Cools *et al.* (9). Briefly, several years ago, 60 male and 60 female rats from a normal Wistar population were injected with 1.5 mg/kg apomorphine, sc, which induced stereotypic gnawing behavior. These rats were divided into 3 groups according to their gnawing response; group A consisted of rats with a low gnawing score (<10/45 min), the apo-unsus rats (27% of the original population); group B consisted of rats with a high gnawing score (>500/45 min), the apo-sus rats (23% of the original population); and group C consisted of rats with a score between 10–500/45 min. Nine pairs of rats in group A and 9 pairs of rats in group B were used to start the breeding lines. After weaning at the age of 30 days, male and female young were separated and grouped together (2 or 3 animals/cage/sex/selection line).

Retention of genetic selection was tested in rats of the first litter of each generation. At the age of 60 days, these rats were injected with apomorphine and tested using the same procedure as that described above. Apo-unsus rats with the lowest scores and apo-sus rats with the highest scores were selected and used for breeding the next generation; brother-sister crossings were avoided. Rats of the second and third litters of each generation were used for the experiments. The mean (\pm SEM) gnawing scores of the rats of the first litters of the 12–14th generation used in this study were 777 ± 75 for apo-sus rats and 53 ± 35 for apo-unsus rats.

Rats were housed under controlled conditions of temperature (23°C) and light (12-h light, 12-h dark cycle; lights on at 0700 h), with food and water available *ad libitum*. After cannulation, rats were housed individually. Animals were randomly assigned to one of the different independent experimental groups. Rats used for receptor binding and *in situ* hybridization experiments did not participate in any other experiment to avoid stress effects on corticosteroid receptor properties.

All experiments were performed in accordance with protocols approved by the animal care committee of the Faculty of Medicine, University of Leiden (Leiden, The Netherlands).

Surgery

On the day of surgery, a silicone cannula (id, 0.5 mm; od, 1.0 mm) was implanted into the right atrium via the external jugular vein, according to the method of Steffens (12). Surgery was performed under Fentanyl (0.01 mg/50 μ l/100 g BW, im; Janssen Pharmaceutica, Tilburg, The Netherlands) and Dormicum (midazolam; 250 μ g/50 μ l/100 g BW; Hoffman LaRoche, Mijdrecht, The Netherlands) anesthesia under sterile conditions. During the 1-week recovery period, blood flow through the cannulas was checked daily, which at the same time permitted habituation of the animals to the sampling procedure.

Bilateral adrenalectomy (ADX) was carried out under ether anesthesia using the dorsal approach. ADX was performed between 0900–1100 h.

Experimental designs

Several blood samples were collected via a chronic jugular venous cannula implanted in rats of the 2 selected lines over a period of maximally 2 h. After the conditioned emotional response, we measured the time course of plasma ACTH [total as well as free plasma corticosterone; apo-sus, $n = 7$; apo-unsus, $n = 8$; 7 blood samples with the first blood sample (basal) taken immediately after connection of the cannula and 1 h before the rat was subjected to the stress test procedures] and plasma PRL (apo-sus, $n = 5$; apo-unsus, $n = 4$; 10 blood samples with the first blood sample at time zero, taken immediately after exposure to the stress stimulus). To control for the effect of the sampling procedure, no stress groups were used ($n = 5$ /group; 7 blood samples and hormone assays as for plasma ACTH above). In additional experiments, plasma ACTH was measured only at 2.5 min during the conditioned emotional response ($n = 5$ /group), and plasma PRL was estimated only 2.5 min after exposure to a novel environment (apo-sus, $n = 4$; apo-unsus, $n = 7$). Basal morning (0900 h) samples of circulating plasma ACTH, total and free corticosterone, and PRL were collected on the day before the stress test (apo-sus, $n = 15$; apo-unsus, $n = 20$). 7) Blood samples collected via the tail vein were used to assess the long term effect of ADX on the morning and evening levels (1700 h) of plasma ACTH ($n = 6$ /group). Animals used for receptor binding and *in situ* hybridization did not participate in any other experiment.

Blood sampling procedures

Blood samples were collected from freely moving rats. The jugular venous cannula was connected to a long polypropylene tube that did not hamper the movement of the animal. Blood samples of 300 μ l were withdrawn using sterile syringes, collected in EDTA-coated tubes, and centrifuged at 6000 rpm for 10 min. The loss of body fluid after each blood sample was compensated for by immediate injection of an equal volume of sterile saline via the cannula. During blood sampling from the tail vein, the rat was loosely held by hand. A small incision was made at the tip of the rat's tail. For each sample, a volume of approximately 300 μ l blood was collected in microvettes (EDTA coated; Sarstedt, Numbrecht, Germany) for a maximum of 90 sec. Plasma was stored at -20°C until assayed for ACTH, total and free corticosterone, and PRL.

Stress tests

Stress tests were run between 0800–1330 h. Two days before testing, the rats were placed in the test cage (50 \times 50 \times 35 cm) for 3 min and returned to their home cage. The next day, the rats were placed into the same test cage, and 10 sec later they received an electric tail shock (0.1 mA for 1 sec); after another 10 sec, the rats were moved to the experimental blood sampling cage (25 \times 25 \times 35 cm). On the third day, the day of blood sampling, the animals were returned to the test cage for 90 sec. This is a conditioned emotional stimulus. Thereafter, they were connected to the blood sampling cannula and moved to the blood sampling cages. Animals in the no stress control group were housed for 1 day in the blood sampling cage and connected to the long tubes 1 h before the withdrawal of blood samples. Rats remained in the blood sampling cage until the last blood sample was collected. For the novelty stress experiment, rats of both lines were transferred at time zero from their home cage to the novel environment of the blood sampling cage.

Determination of ACTH

Plasma ACTH immunoreactivity was measured by RIA, using a commercially available ACTH antibody directed against ACTH-(5–18) (IgG Corp., Nashville, TN). The RIA was based on the method previously described (10, 13), with minor modifications. ACTH-(1–39) was used as a standard, and [125 I]ACTH (1–39) as a tracer. The tracer and sheep antirabbit IgG were obtained from Prof. Th. Benraad, University of Nijmegen (Nijmegen, The Netherlands). Aliquots of plasma samples (50 and 25 μ l) were used instead of 100 μ l supernatant.

Determination of total and free corticosterone

Plasma corticosterone was measured by RIA using an antiserum raised against corticosterone-21-hemisuccinate BSA (Prof. Th. Benraad, University of Nijmegen), as described previously (14). The sensitivity of the corticosterone RIA is 0.01 μ g/dl sample.

Free corticosterone was determined in a 200- μ l pooled plasma sample (from six to eight rats, 25–30 μ l/rat)/time point. The bound fraction was separated from free corticosterone by ultrafiltration (GPR centrifuge with a fixed angle rotor, Beckman, Palo Alto, CA), as described previously (15). The MPS-1 ultrafiltration device (Amicon Corp., Danvers, MA) contained a YMT membrane and a polyethylene cup to collect the filtrate. During centrifugation, which lasted for 15 min (4000 rpm; 1700 \times g), the temperature within the ultrafiltration device was kept at 37 ± 0.1 C. The supernatant (± 150 μ l) contained the unbound free corticosterone, which was measured in the RIA described above for total corticosterone. Each measurement was performed in triplicate.

Determination of plasma PRL

Plasma PRL was assayed by a homologous double antibody method using the NIDDK rat PRL hormone kit provided by the Rat Pituitary Hormone Distribution Program. The rat PRL standard used was rat PRL RP-3.

Tissue collection

Apo-sus and apo-unsus rats were killed by decapitation between 0900–1300 h. Brains were rapidly frozen in isopentane at -60 C, sectioned in a cryostat (coronal plane, 20 μ m), and mounted on poly-L-lysine (Sigma Chemical Co., St. Louis, MO)-coated slides. Sections of the dorsal hippocampus and the hypothalamic paraventricular nucleus and arcuate nucleus were collected according to the atlas of Paxinos and Watson (16).

TH in situ hybridization

A synthetic oligo encoding rat TH (45 nucleotides, 1442–1487) was prepared on an Applied Biosystems DNA synthesizer (Foster City, CA). The probe was 3'-end labeled using [α - 35 S]deoxy-ATP (SA, >1000 Ci/mmol; Amersham) and terminal deoxynucleotidyltransferase (Boehringer Mannheim, Indianapolis, IN). The hybridization procedure described by Nicot *et al.* (17) was used.

Sections taken at the level of the arcuate nucleus were fixed with 4% formaldehyde in PBS, pH 7.4, for 10 min and rinsed twice in PBS and once in $4 \times$ SSC (standard saline citrate). Subsequently, the sections were placed in 0.25% acetic anhydride in 0.1 M triethanolamine for 10 min to reduce nonspecific binding. This prehybridization procedure was completed with a rapid wash with water and dehydration in ethanol and chloroform. The sections were subsequently air dried. The probe was diluted in a hybridization buffer containing 50% formamide, $4 \times$ SSC, $1 \times$ Denhardt's solution, 1% sarcosyl, 10 mM dithiothreitol, 0.1 M potassium phosphate (pH 7.4), 250 μ g/ml yeast transfer RNA, 250 μ g/ml herring sperm DNA, and 100 μ g/ml polyadenylic acid. Hybridization buffer (85 μ l) was applied to each slide and sealed with a coverslip and rubber cement. For overnight hybridization, performed at 42 C, the slides were placed in moist containers to prevent dehydration. The next day, the coverslips were removed, and the sections were washed at 45 C in $4 \times$ SSC containing 0.02% SDS for 30 min, twice in $1 \times$ SSC for 30 min, and twice in $0.1 \times$ SSC for 30 min each time. Finally, the sections were dehydrated in ethanol and air-dried before being exposed to a

Kodak X-Omat AR film (Eastman Kodak, Rochester, NY) for 14 days at room temperature. The films were used for quantification, as described below, and distribution analysis. Control sections were treated with ribonuclease A (40 μ g/ml; 30 min at 37 C) before hybridization with the TH probe.

In situ hybridization using RNA probes encoding MR and GR mRNA

Different 35 S-labeled complementary RNA antisense probes were employed to hybridize with complementary brain tissue MR and GR mRNA. The antisense and sense MR probes were transcribed from a 513-bp rat brain complementary DNA (cDNA) fragment, which encodes for the last 30 amino acids at the C-terminus of MR plus the adjacent highly specific 3'-untranslated region (courtesy of J. L. Arriza, San Diego, CA). The antisense and sense GR probes were transcribed from a 500-bp cDNA fragment (courtesy of M. C. Bohn, Rochester, NY), subcloned from a 2.8-kilobase fragment of the rat liver GR cDNA (courtesy of K. R. Yamamoto, San Francisco, CA) and encoding for the N-terminal region of the GR molecule. Tissue sections were fixed with 4% paraformaldehyde in PBS, pH 7.4, for 10 min. The prehybridization and subsequent hybridization procedures described previously for adult brain tissue (18) were used without further modifications. A 90- μ l hybridization mix at a probe concentration of 5×10^7 cpm/ml was applied to each slide; the slide was subsequently coverslipped. At a final stage, the sections were exposed for 6 days to a Kodak X-Omat AR film for semiquantification. The slides were then dipped in Kodak NTB-3 emulsion (diluted 1:1 in 0.6 M ammonium acetate at 43 C) and exposed for another 2–3 weeks. The emulsion-coated sections were used to confirm the cellular localization of the hybridization signal. The control study consisted of hybridization with the receptor-specific MR and GR sense complementary RNA probes.

In vivo autoradiography

Five apo-sus and five apo-unsus male rats were injected with 100 μ Ci [3 H]corticosterone/100 g BW via a cannula in the jugular vein 24 h after ADX (19). Sixty minutes later, the rats were decapitated, and the brains were frozen on dry ice, sectioned in a cryostat (coronal plane, 30 μ m), and mounted on slides. Trunk blood was collected in heparin-coated tubes to check plasma [3 H]corticosterone levels. The sections were exposed to 3 H-sensitive Ultrafilm (Leica, Nusslock, Germany) for 4 months. Nonspecific binding of [3 H]corticosterone was determined by pretreating the rat with a 500-fold excess of unlabeled corticosterone.

In vitro radioligand binding to cytosolic MR and GR

Rats were decapitated 24 h after ADX. Hippocampi were dissected out, frozen on dry ice, and stored at -80 C until assay. A total of 3 independent experiments were carried out using pooled samples of hippocampal cytosol in accordance with a previously established method (11, 19, 20) with minor modifications. Briefly, aliquots of the cytosol were incubated with a wide range of concentrations (0.3–20 nM) of [3 H]aldosterone (SA, 85.0 Ci/mmol; New England Nuclear, Boston, MA) in the presence of a 100-fold molar excess of RU28362 to determine MR binding or [3 H]RU28362 (SA, 77.5 Ci/mmol; Roussel-UCLAF Pharmaceuticals, Romainville, France) to determine binding to GR. Nonspecific binding was determined by the inclusion of a 1000-fold molar excess of the appropriate unlabeled steroid in parallel samples. Each of the 3 assays was carried out in duplicate and consisted of tissue from 6–10 animals. After a 16-h incubation at 2 C, the bound fraction was separated from the free fraction by means of a column chromatography method using Sephadex LH-20 minicolumns (Pharmacia Fine Chemicals, Uppsala, Sweden). The cytosolic protein content was determined using the method of Lowry *et al.* (20, 21). All binding data were calculated in accordance with the method of Scatchard to determine the apparent binding affinity (K_d) and the maximum binding capacity (B_{max}).

Computer-assisted image analysis

Optical density was quantified with an Olympus image analysis system (Paes, The Netherlands) equipped with a Cue CCD camera. Film

background was subtracted after shading correction. For the *in vivo* autoradiography experiments, the optical densities were quantified on the basis of a standard curve calculated from a set of ^3H microscales (Amersham, Aylesbury, UK). For TH mRNA quantification, a set of ^{14}C microscales, which was comparable with a ^{35}S microscale (Amersham), was used. From each brain, 6–10 sections were measured by outlining the different hippocampal subfields, for MR and GR, or the arcuate nucleus, for TH mRNA.

Statistics

Stress-induced plasma hormone data were evaluated using one-way ANOVA for repeated measurements (factor group; apo-sus and apo-unsus rats) and *post-hoc* Tukey's highest significant difference test (statistical package of SYSTAT). Basal plasma hormone levels, ACTH levels after ADX, TH mRNA, MR and GR mRNA, and *in vivo* and *in vitro* binding data were evaluated by Student's *t* test. Significance was taken at $P < 0.05$. Data are presented as the mean \pm SEM.

Results

Plasma PRL

The conditioned emotional stimulus evoked significantly different PRL release in both groups [$F_{(1,7)} = 30.85$; $P < 0.001$; Fig. 1A]. Apo-unsus rats responded with a pronounced increase in plasma PRL concentrations, whereas apo-sus rats showed no stress-induced response throughout the sampling period [time effect, $F_{(9,63)} = 5.82$; $P < 0.001$; time \times group interaction, $F_{(9,63)} = 3.23$; $P < 0.01$]. PRL levels measured in blood samples obtained 2.5 min after exposure to a novel environment showed similar differences between the groups (Fig. 1B); apo-unsus rats showed a significantly higher plasma PRL level than apo-sus rats. Under basal conditions, plasma PRL levels did not differ between the rat lines ($P = 0.10$; Table 1).

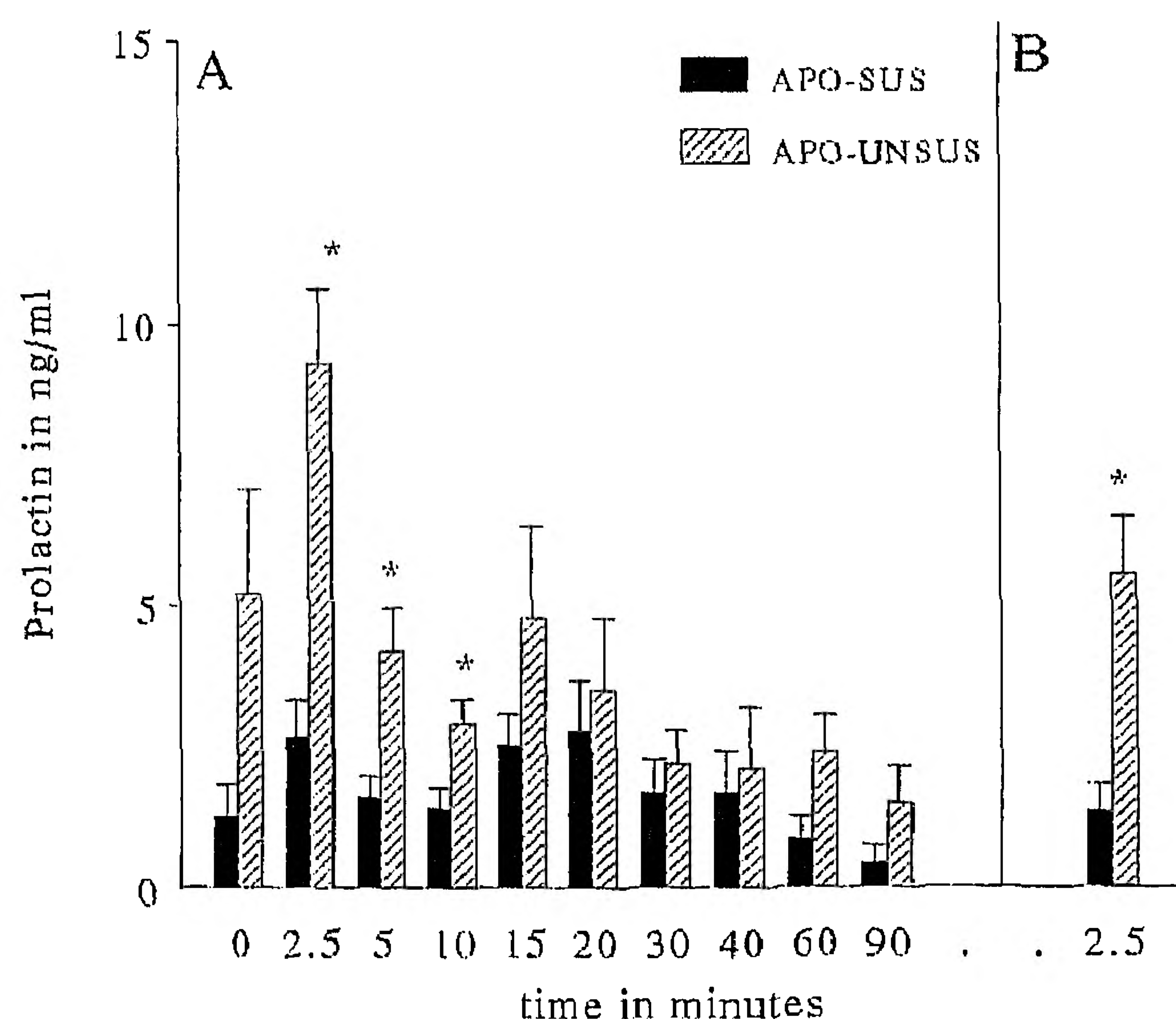


FIG. 1. Time course of plasma PRL levels determined in blood samples collected during a conditioned emotional response (A; apo-sus, $n = 5$; apo-unsus, $n = 4$) and 2.5 min after exposure to novelty (B; apo-sus, $n = 4$; apo-unsus, $n = 7$). Data represent the mean \pm SEM of two independent experiments. *, $P < 0.05$, apo-sus vs. apo-unsus rats.

TABLE 1. Basal morning ACTH (picograms per ml), total and free corticosterone (micrograms per dl), and PRL (nanograms per ml) in plasma

	Apo-sus	Apo-unsus
ACTH	105 \pm 6.1 (n=15)	79.3 \pm 3.1 ^a (n=20)
Total corticosterone	1.5 \pm 0.2 (n=12)	1.4 \pm 0.2 (n=24)
Free corticosterone	0.12 \pm 0.02 (n= 9)	0.20 \pm 0.04 ^b (n= 9)
PRL	0.66 \pm 0.2 (n= 6)	1.58 \pm 0.44 (n= 7)

Data are expressed as the mean \pm SEM.

^a $P < 0.01$ compared to the same measure in apo-sus rats.

^b $P < 0.05$ compared to the same measure in apo-sus rats.

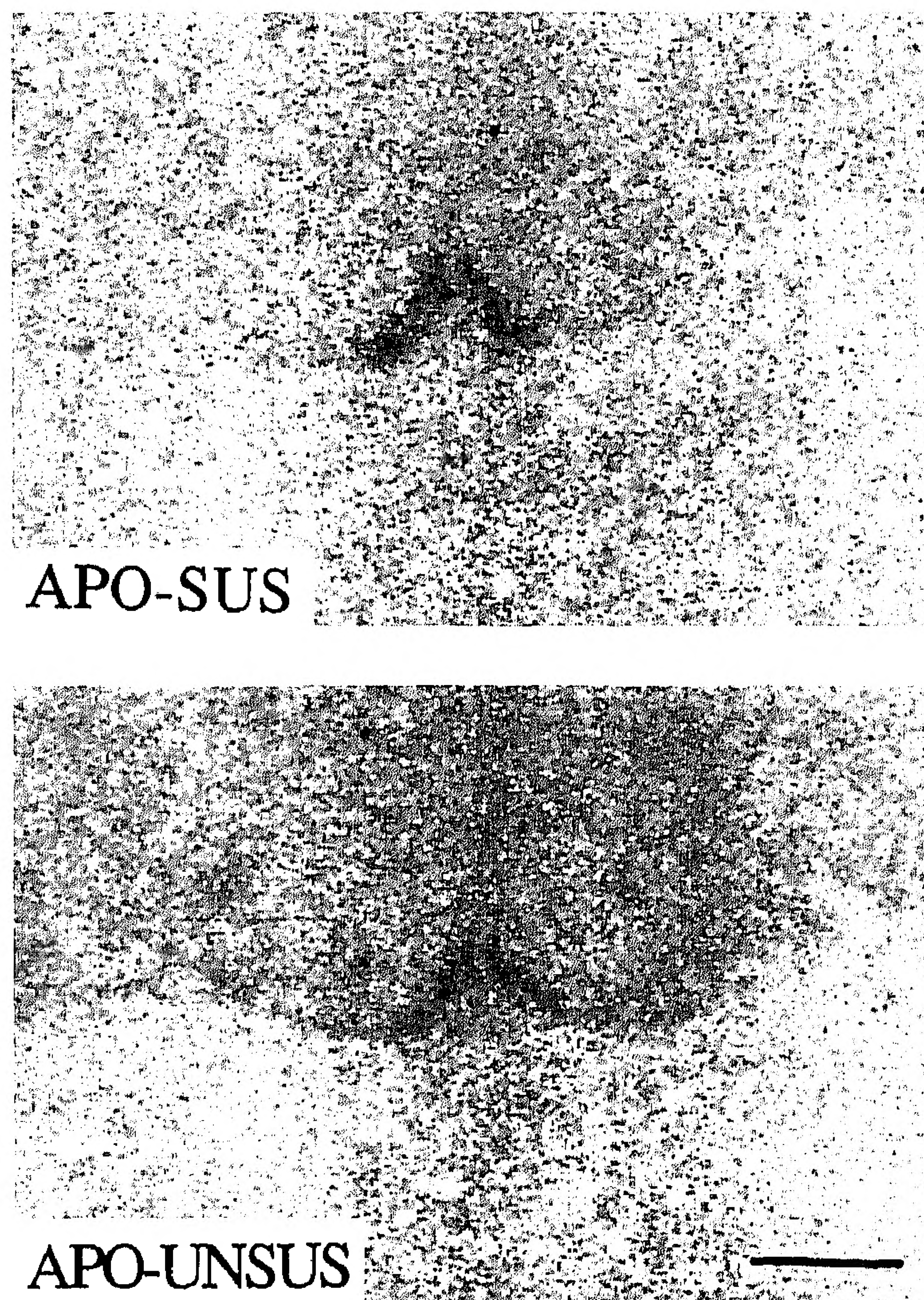


FIG. 2. Photomicrograph of brain sections labeled *in situ* with a TH oligonucleotide probe. Levels of TH mRNA found in the arcuate nucleus of apo-sus are higher than those in apo-unsus rats. Scale bar = 1 mm.

TH gene expression

TH mRNA expression in the nucleus arcuatus was significantly higher in apo-sus than in apo-unsus rats (Fig. 2). Quantification of the autoradiograms resulted in 91.6 ± 4.5 and 63.6 ± 2.7 arbitrary units of optical density for apo-sus and apo-unsus rats, respectively ($P < 0.01$).

ACTH and corticosterone in plasma: basal levels and response to a conditioned emotional stimulus

Under basal morning conditions, plasma ACTH levels were significantly elevated, total corticosterone plasma lev-

els were similar, and free corticosterone plasma levels were significantly reduced in apo-sus rats compared with apo-unsus rats (Table 1).

Exposure to the conditioned emotional stimulus evoked a significantly different ACTH response in apo-sus compared with apo-unsus rats [$F_{(1,13)} = 6.35$; $P < 0.05$]. The stress response occurred in both groups [time effect, $F_{(6,78)} = 50.71$; $P < 0.001$; Fig. 3A] with a significantly different time course [time \times group interaction, $F_{(6,78)} = 3.07$; $P < 0.01$]. Peak ACTH levels, which were measured in a separate experi-

ment, were reached by 2.5 min after stress and were similar in both groups (mean \pm SEM, 779 ± 275 and 743 ± 154 pg/ml ACTH in apo-sus and apo-unsus rats, respectively). Calculation of the increase from the basal level of ACTH resulted in a similar picture of prolonged ACTH release in the apo-sus group [change in ACTH (mean \pm SEM) at 15 and 30 min for apo-sus rats, 496 ± 69 and 328 ± 29 pg/ml; for apo-unsus rats, 216 ± 46 and 80 ± 14 pg/ml]. Inspection of the data with respect to the significant time \times group interaction revealed that plasma ACTH levels remained elevated for a longer period of time in the apo-sus group compared with those in the apo-unsus group.

Total corticosterone plasma concentrations, measured in the same animals, showed no difference between rat groups. Within the first 15 min after stimulation, corticosterone levels increased significantly in both groups until comparable peak levels were reached [time effect, $F_{(6,78)} = 25.19$; $P < 0.01$; Fig. 3B]. However, determination of free corticosterone plasma concentrations did show a different effect (Fig. 3C). Blood samples collected from apo-sus rats 15 and 30 min after conditioned emotional stimulation showed twice as much free corticosterone as those obtained from apo-unsus rats at the same time points. Statistical analysis could not be performed on these data because determination of free corticosterone in pooled samples of six to eight rats resulted in a single value per time point for an experimental condition.

Rats of the no stress control groups did not show an increase above basal levels in ACTH or total and free corticosterone levels over the time period tested (data not shown).

Plasma ACTH concentrations after ADX

Stress-induced ACTH levels reached significantly higher values in apo-sus rats. ADX eliminates the feedback inhibition of corticosterone on ACTH release and produces, after a longer time interval, a situation in which the maximal synthetic capacity of ACTH by pituitary corticotrophs can be measured. Table 2 shows that ADX resulted in increased plasma ACTH levels in rats of both lines. This increase was only significantly higher in apo-sus rats compared to that in apo-unsus rats 6 h after ADX. Plasma ACTH levels were similar in both groups 16 days after ADX.

In vivo autoradiography

In 1-day adrenalectomized rats, *in vivo* corticosteroid receptor binding was measured after the administration of a tracer dose of [3 H]corticosterone (1.4 nmol/rat). This concentration is sufficient to half-maximally occupy MR, but is still too low to result in significant GR occupancy (19, 20). Autoradiograms of *in vivo* uptake of [3 H]corticosterone 60 min after the injection revealed a significantly higher retention of radioactively labeled corticosterone in apo-sus than in apo-unsus rats (Fig. 4). Plasma radioactivity levels at 60 min were similar (mean \pm SEM, $31,600 \pm 2,688$ and $29,042 \pm 1,741$ dpm in apo-sus and apo-unsus groups, respectively).

Radiolabeled corticosterone was retained predominantly by the neuronal cell nuclei of the hippocampus; measurement of the different cell fields of the dorsal hippocampus indicated a significant increase in pyramidal cell fields CA₁

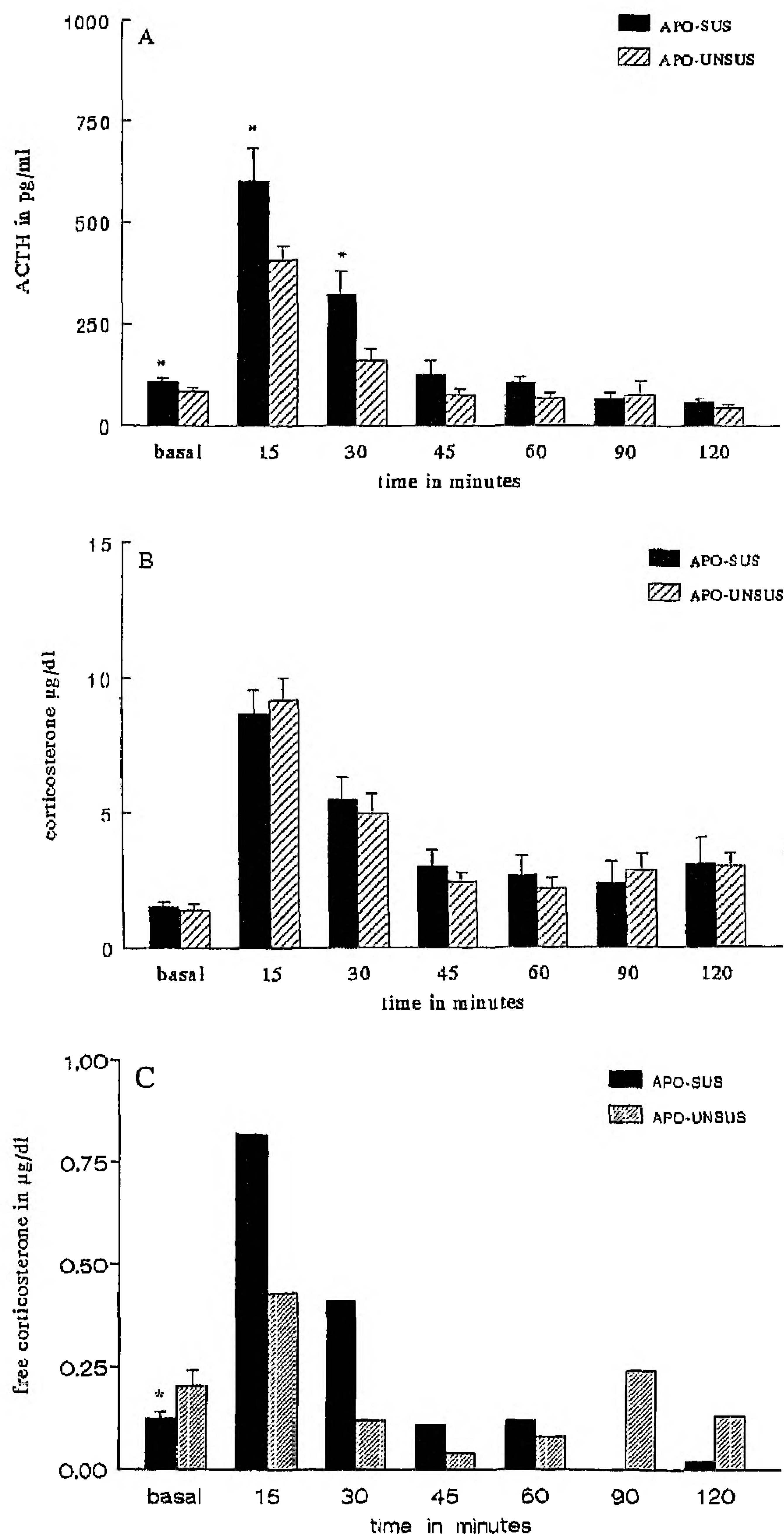


FIG. 3. Plasma ACTH (A), corticosterone (B), and free corticosterone (C) levels in apo-sus ($n = 7$) and apo-unsus ($n = 8$) rats during a conditioned emotional response. Data represent the mean \pm SEM of four independent experiments with rats of two different generations. *, $P < 0.05$ compared to apo-unsus rats.

TABLE 2. Plasma ACTH levels (picograms per ml) in apo-sus (n = 5–6) and apo-unsus (n = 6) rats at different time points after adrenalectomy

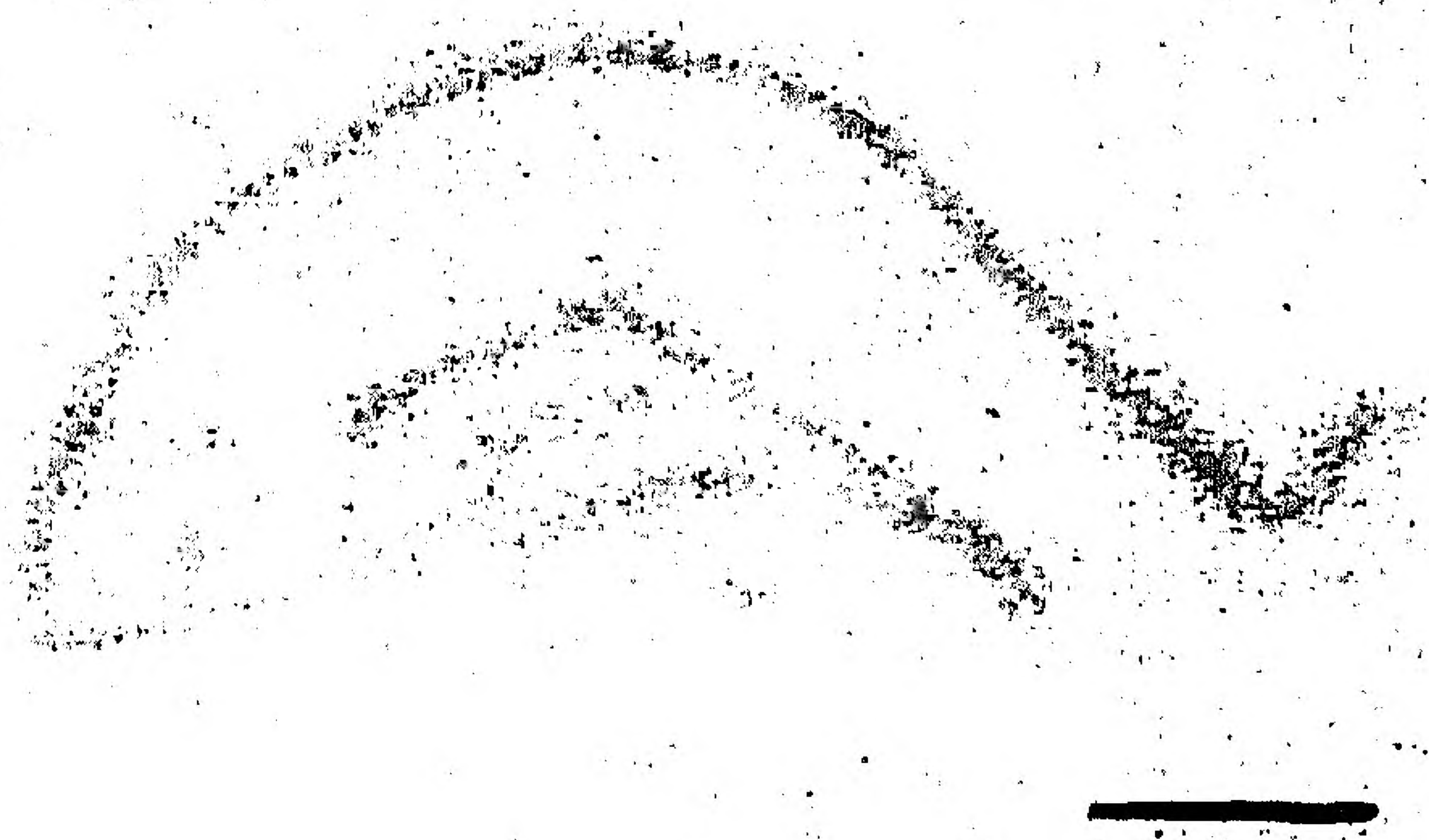
	Apo-sus		Apo-unsus	
	am	pm	am	pm
Before ADX	105 ± 6	225 ± 27	79.3 ± 3 ^a	140 ± 10 ^a
Time after ADX				
6 h	1001 ± 127		584 ± 48 ^a	
1 day	541 ± 66	1621 ± 570	827 ± 142	1320 ± 71
8 days	3098 ± 196	4757 ± 1009	4172 ± 297	5689 ± 831
16 days	2619 ± 915		3174 ± 929	

^a *P* < compared to the same measure in apo-sus rats.^b *P* < 0.05 compared to the same measure in apo-sus rats.

APO-SUS



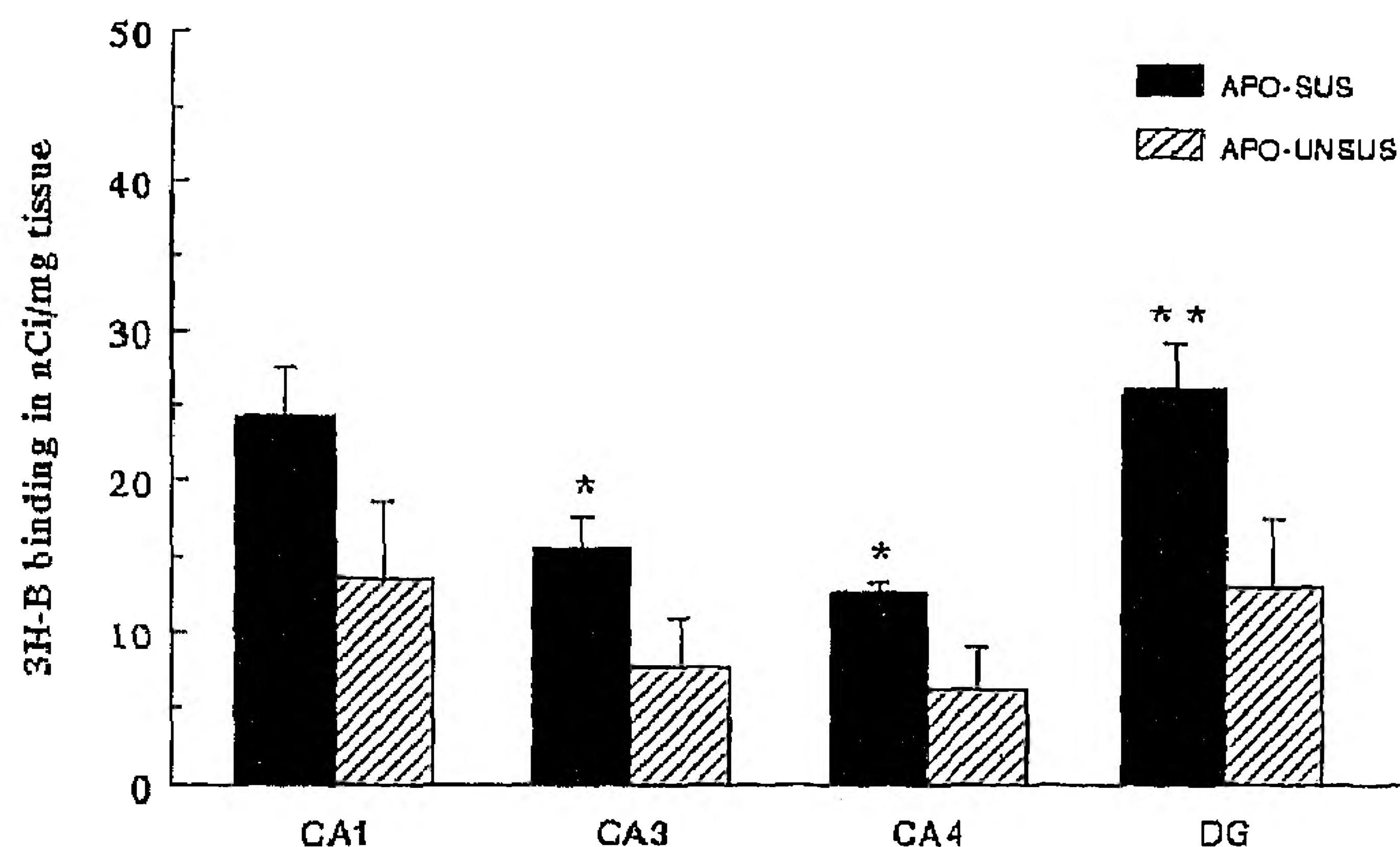
APO-UNSUS

**FIG. 4.** [³H]Corticosterone uptake in the dorsal hippocampus of apo-sus (A) and apo-unsus (B) rats injected 1 h previously with a tracer dose (50 μCi/100 g BW, iv) in adrenalectomized rats. Scale bar = 1 mm.

(39.9%), CA₃ (43.9%), and CA₄ (41.9%) and in the granular cells of the dentate gyrus (45.8%) in apo-sus rats compared with levels in apo-unsus rats (Fig. 5).

In vitro cytosol binding

Apo-sus rats showed a significantly larger hippocampal MR binding capacity than apo-unsus animals (*P* < 0.05; Table 3), whereas GR capacity did not differ between the groups.

**FIG. 5.** Difference in retention of [³H]corticosterone in rat brain sections of adrenalectomized apo-sus (n = 4) and apo-unsus (n = 4) rats. Data are expressed as the mean ± SEM in nanocuries per mg wet tissue (10–12 sections/rat were measured). *, *P* < 0.05; **, *P* < 0.01 (compared to apo-unsus rats).**TABLE 3.** Binding constants of MRs and GRs in the hippocampal cytosol of apo-sus and apo-unsus rats

	Apo-sus	Apo-unsus
MR B _{max} (fmol/mg protein)	158.8 ± 19.74 ^a	106.7 ± 10.0
K _d (nM)	1.02 ± 0.04	0.83 ± 0.23
GR B _{max} (fmol/mg protein)	306.4 ± 46	275.4 ± 35.9
K _d (nM)	1.98 ± 0.51	1.78 ± 0.48

Values are the mean ± SEM of 3 independent assays, each carried out in duplicate (6–10 rats/assay).

^a *P* < 0.05, apo-sus vs. apo-unsus rats.

The apparent binding affinity (K_d) of MR and GR did not differ between apo-sus and apo-unsus groups (Table 3).

MR and GR gene expression

Figure 6 shows a representative photomicrograph of ³⁵S-labeled MR mRNA and GR mRNA in the hippocampus of apo-sus rats. Quantitative image analysis of the autoradiograms showing hybridization of specific ³⁵S-labeled anti-sense probes with MR and GR mRNA in brain sections revealed no significant difference in the hippocampus or PVN between the rat lines (data not shown).

Discussion

The present study shows that genetic selection for apomorphine susceptibility results in two rat lines exhibiting

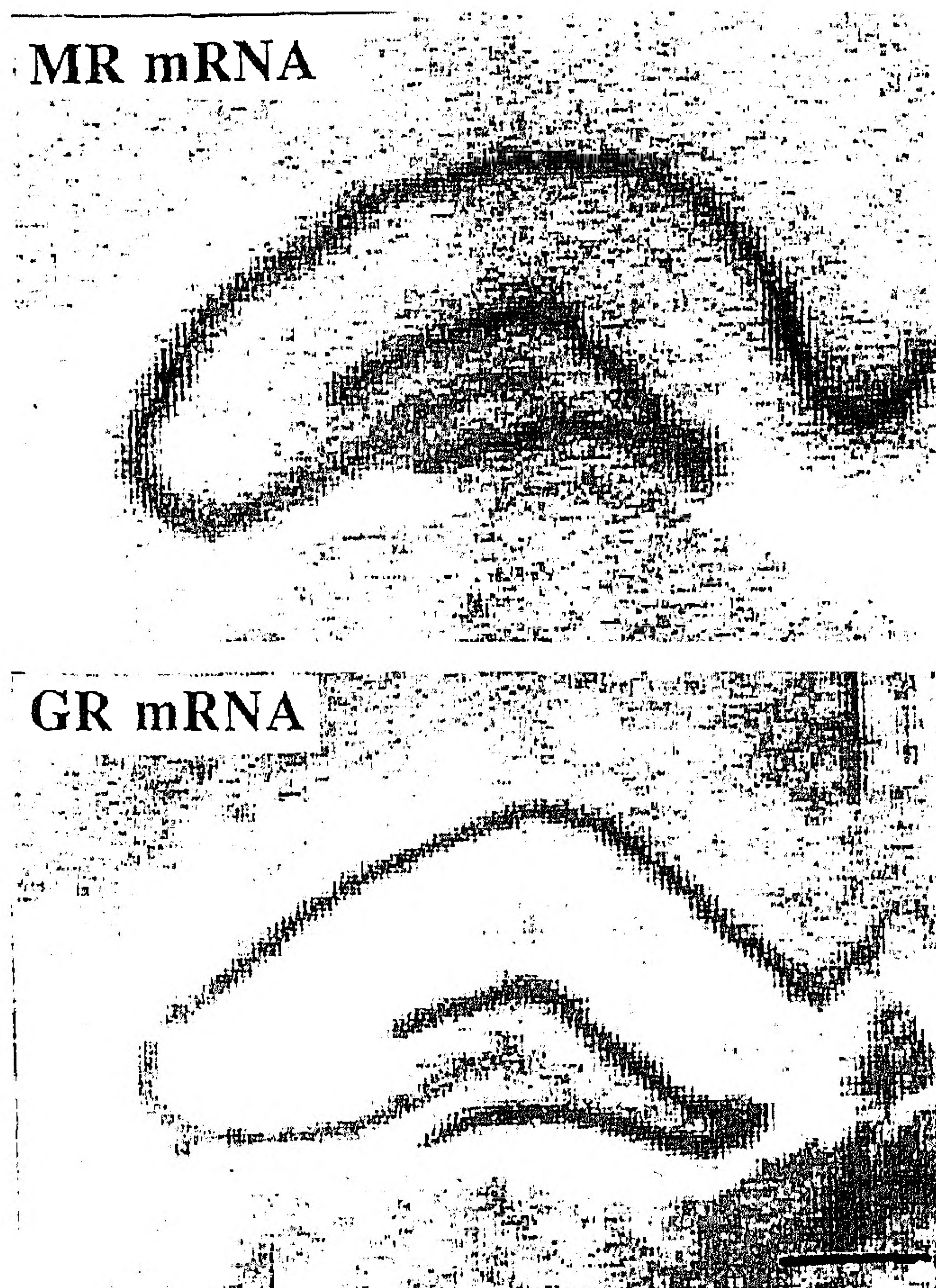


FIG. 6. Representative photomicrograph of brain sections labeled *in situ* with a MR or GR antisense probe. Scale bar = 1 mm.

divergent ACTH, corticosterone, and PRL responses. Collectively, these data show that apo-sus rats have higher and prolonged stress-induced responses of ACTH and free corticosterone than rats of the apo-unsus line, whereas the total corticosterone level is not different in the two rat lines. In contrast, stress-induced PRL release in apo-sus rats is attenuated. Thus, these rat lines, with distinct dopamine phenotypes, show pronounced differences at multiple levels of endocrine regulation.

The rat lines were genetically selected on the basis of extreme differences in apomorphine-induced stereotypical gnawing behavior. Further behavioral studies showed that the selected lines represent individuals coexisting in a normal unselected rat population that display profound differences in coping with stress. In the face of the psychosocial challenge of defeat, the apo-sus rats displayed a fleeing response, rather than the immobility (freezing) response of their counterparts (9). The present study suggests that these extreme differences in coping with stress coincide with the divergence in PRL and pituitary-adrenal responses. Using genetic selection, we found a linkage of this divergence in features of the stress response system with dopamine phenotype.

Apo-sus rats display a strongly inhibited stress-induced PRL release. This blockade of PRL suggests an enhanced

inhibitory control exerted by dopamine released from A12 neurons. Indeed, the level of TH mRNA was significantly higher in the A12 dopaminergic region of the apo-sus rats. Naturally, caution should be exercised when interpreting the functional implications of TH mRNA levels until other indexes, such as TH enzyme activity or the actual dopamine release, have been measured. However, the TH mRNA level in the A9 substantia nigra cells is also elevated in apo-sus rats, where the level of TH mRNA in A10 is not different between the two rat lines. Moreover, the apo-sus animals showed significantly more [125 I]iodosulpiride binding to D2 receptors in the striatum, but not in the nucleus accumbens, of the apo-sus animals (22). Thus, selection for apomorphine susceptibility is related to altered dopaminergic function in the tuberoinfundibular and nigrostriatal pathways. These dopaminergic systems are associated with control of PRL release and stereotypical behavior, respectively.

Although the attenuation of stress-induced PRL release in apo-sus rats seems to be associated with a higher level of infundibular dopaminergic activity, the mechanism underlying elevated ACTH levels in these same animals is different. ACTH release by the anterior pituitary is not under the direct control of dopamine, but stimulatory actions of dopamine on CRH neurons have been reported (7). Previously, we observed that under basal conditions, CRH mRNA in the PVN is higher in apo-sus rats (10). Therefore, it is conceivable that an increased dopamine reactivity is related to the reported elevation in CRH mRNA levels and subsequently ACTH release at the time that PRL is inhibited. Indeed, such a stimulatory role of the dopamine system on CRH release has been suggested, as basal and stress-induced corticosterone levels were reduced after neurotoxic lesioning of the mesencephalic dopamine neurons with 6-hydroxydopamine (23).

In apo-sus rats, basal ACTH levels were elevated at the circadian trough and peak. Although their basal corticosterone levels are not different, the fraction of free corticosterone is reduced. Accordingly, the rat lines differ in the apparent set-point of basal HPA activity. The elevated basal ACTH level in apo-sus rats persists in the first 6 h post-ADX, but subsequently disappears at a time when genomic control exerted by corticosterone presumably wears off. At post-ADX intervals exceeding 24 h, when genomic corticosterone effects are eliminated, circadian changes in ACTH level were not different between the two rat lines, indicating a difference in the regulation of ACTH release rather than in the capacity to synthesize ACTH.

The relatively higher ACTH and the similar corticosterone levels in apo-sus rats suggest hyporesponsiveness of the adrenal cortex to ACTH. This adrenal hyporesponsiveness seems to be due to a reduction in synergizing adrenal factors (10), among which CRH, vasoactive intestinal polypeptide, and acetylcholine have been best documented (24–26), although the influence of catecholamines and altered sympathetic tone cannot be excluded (10). Thus, the line differences in ACTH/corticosterone reciprocity suggest that adrenal responsiveness is a critical variable in the set-point regulation of the pituitary-adrenal system.

The levels of free corticosterone are elevated in apo-sus rats 30 and 45 min after conditioned emotional stimulation.

This time-dependent difference in free corticosterone after stress is based on a single measurement of pooled aliquots obtained by sequential blood sampling of six to eight animals and validates a similar finding after exposure of the rats to the stress of a novel environment (10). Although the stress-induced level of free corticosterone is higher in apo-sus rats, their basal free corticosterone level is lower than that in their apo-unsus counterparts. These peculiar line differences suggest a critical role of corticosteroid-binding globulin binding in the free hormone level and support evidence that corticosteroid-binding globulin is not a constitutively produced liver protein but, rather, a dynamic component in HPA regulation (27).

The finding that ACTH levels are significantly higher in apo-sus rats at 15 and 30 min indicates a prolonged ACTH response, as the peak ACTH levels reached 2.5 min after stress show no difference between the rat strains. This sustained elevation of ACTH and free corticosterone levels in apo-sus rats suggests a greater corticosteroid feedback resistance relative to that in their apo-unsus counterparts. Moreover, dexamethasone suppression and CRH challenge showed no strain differences, indicating that feedback resistance resides at a central, rather than a pituitary, site (10). Corticosteroid action in the brain on the regulation of HPA activity involves binding to two types of corticosteroid receptors, *i.e.* MR and GR (28–33). The present study shows that the corticosteroid feedback resistance did not coincide with altered GR mRNA in hippocampus and parvocellular neurons of the PVN. MR mRNA was also not different in any of the hippocampal subfields.

Administration of radiolabeled corticosterone to apo-sus rats 24 h after ADX, however, resulted in an increased retention of the ligand in hippocampal neurons. The very low dose of radiolabeled corticosterone given to the adrenalectomized rats is sufficient to half-maximally occupy the high affinity MR, but it is still insufficient for significant occupation of the lower affinity GRs (20, 34). This increased *in vivo* binding is supported by the finding that apo-sus rats had a 50% rise in MR B_{max} measured *in vitro* in hippocampal cytosol (11). As the MR mRNA levels are comparable, the difference in apomorphine susceptibility is apparently paralleled by site- and receptor-specific changes in translation, receptor processing, or posttranslational events such as phosphorylation. These posttranscriptional processes seem to result, in some brain areas such as the hippocampus, in a larger number of MR-binding sites. It could well be that the higher MR capacity is an adaptive response to the lower basal free corticosterone level circulating in apo-sus rats.

Several recent studies have pointed to an interaction among dopamine, brain corticosteroid receptors, and the HPA axis (4, 6–8, 23, 35–37). The present study also shows that dopamine phenotype is related to basal and stress-induced activities of the HPA axis. The apo-sus rats are marked by hyporesponsive adrenals, lower basal free corticosterone levels, and increased hippocampal MR levels, which participate in the control of basal HPA activity. Apo-sus rats display feedback resistance in neuroendocrine regulation, but no strain differences in GR binding and mRNA were observed. Thus, our findings suggest a reduced containment of the stress response system by corticosterone in

apo-sus rats. However, the feedback resistance also results in increased levels of free circulating corticosterone. Such a sustained elevation of bioactive corticosterone is thought to exert a GR-dependent facilitating and sensitizing effect on ascending dopaminergic neurons (6, 37, 38). Such an enhanced dopamine drive to the CRH neurons is associated with a condition of feedback resistance and may also explain the lack of PRL response to stress.

In conclusion, using genetic selection, we found a linkage between stress responsiveness and dopamine phenotype. The suggests that individuals with extreme divergence in dopamine, PRL, and ACTH responses coexist in unselected rat populations. It seems that the common denominator for these individual differences is the control exerted by corticosteroids. The rat lines selected for distinct differences in dopamine phenotype, therefore, represent an excellent model to study the mechanism underlying the individual variation in dopamine-dependent vulnerability for psychopathology, such as drug-seeking behavior and psychosis, which may be precipitated by chronic stress and hypercortisolism.

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